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BRIEF COMMUNICATION

Decreased dendritic cell numbers but increased TLR9-mediated interferon-alpha production in first degree relatives of type 1 diabetes patients

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KEYWORDS
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Abstract Objective: Dendritic cells (DCs) play an important role in pathogenesis of autoimmunity, including type 1 diabetes (T1D). In this study, we investigated DC subpopulations and their responses to TLR stimulation in T1D patients and their relatives.
Methods: We analyzed the frequency of myeloid (mDCs) and plasmacytoid DCs (pDCs) in 97 T1D patients (69 onset, 28 long-term), 67 first-degree relatives, and 64 controls. We additionally tested the IFN-alpha production by pDCs upon stimulation with TLR 7, 8 and 9 agonists.
Results: A lower number of mDCs and pDCs were found in T1D patients and their relatives. Of all the tested TLR ligands, only stimulation with CpG 2216 induced IFN-alpha production that was the highest in T1D relatives, except of autoantibody-negative relatives bearing the protective haplotypes.

Abbreviations: T1D, type I diabetes; DC, dendritic cell; mDC, myeloid dendritic cell; pDC, plasmacytoid dendritic cell; TLR, toll-like receptor; IFN, interferon; PBMCs, peripheral blood mononuclear cells.

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Conclusion: Our data demonstrate disturbances in DC number and function expressed most significantly in T1D relatives and point to a potential role of TLR9-induced IFN-alpha production in T1D development.

1. Introduction

Type 1 diabetes is a T-cell-mediated autoimmune disease characterized by known genetic risk factors that involves the slow and progressive destruction of pancreatic islet beta cells and the loss of the ability to secrete insulin. Both genetic background, particularly an association with a high risk HLA DR and DQ haplotypes [1–4], and environmental factors have major influences on the immune mechanisms associated with the disease pathogenesis [5–7].

Over the last two decades, studies addressing the pathogenesis of type 1 diabetes have focused primarily on the role of adaptive immunity [7,8], demonstrating the direct role for T lymphocytes in the destruction of pancreatic islet cells and for B lymphocytes in autoantibody production and defective B cell tolerance [9,10]. Recent studies, however, have suggested an important role for the innate immune system in the development of type 1 diabetes [7,11]. Dendritic cells (DCs) stand at the interface between innate and adaptive immunity and appear to play an important role in the development of type 1 diabetes [12,13]. DCs determine the direction of immune responses by presenting antigen and by influencing the microenvironment through local contacts and the secretion of an array of cytokines, [12]. The capability of DCs to regulate immune environment is particularly important in autoimmune diseases like type 1 diabetes.

DCs presenting beta-cell antigens have been observed in pancreatic islets and lymph nodes in animal models [14]. The transfer of conventional DCs presenting diabetes specific antigens has been shown to initiate diabetogenic response in mice [15]. A similar effect has also been described for plasmacytoid DCs (pDCs), a cell type that serves as a very potent source of type I interferons and has also been shown to promote the development of autoimmune diabetes in mice [16]. Viral infections, particularly enteroviruses, have for a long time been considered as contributors to the pathogenesis of type I diabetes. In this regard, pDCs represent a particularly relevant population as they express toll-like receptors (TLRs) 7–9, which are involved in the recognition of viral infections. It is, however, noteworthy that both types of DCs may also have protective roles under certain circumstances [17,18]. The accumulation of animal model data on the crucial role of DCs in the development of diabetes has promoted similar studies in humans [19]. Due to the apparent difficulties in obtaining DCs from the pancreas or draining lymph nodes in humans, these studies have mostly focused on peripheral blood DCs. There is, however, a considerable discrepancy regarding the status of DCs observed in human cohorts with both increased and decreased DC numbers reported in type 1 diabetes patients [20–25] and thus a further investigation on the role of DCs in type 1 diabetes is warranted.

In this study, we investigated the frequency and functional characteristics of DC subsets in well-defined cohorts of diabetic patients and their first-degree relatives at risk of developing type I diabetes. We attempted to gain an insight into the characteristics of the DC compartment at various stages of type I diabetes pathogenesis, including in autoantibody-negative relatives with a protective genotype, autoantibody-positive individuals at high risk of type I diabetes, newly diagnosed patients, and stabilized patients on insulin replacement therapy.

2. Materials and methods

2.1. Subjects

DC subsets were analyzed from the peripheral blood of 97 patients with type 1 diabetes (69 patients with recent type 1 diabetes onset and 28 long-term treated), and 67 subjects that were first-degree relatives of the type 1 diabetes patients followed in our outpatient department. The control group with negative personal and familial history of autoimmune disease consisted from 64 patients admitted for small surgery (inguinal hernia or knee arthroscopy). Characteristics of these groups are summarized in Table 1.

Patients with recent type 1 diabetes onset were analyzed within seven days following type 1 diabetes diagnosis. Blood collection was performed after the stabilization of clinical status and biochemical parameters such as ions, blood osmolality, and glycemia. At the time of diagnosis, none of subjects had severe diabetic ketoacidosis defined as pH $<$7.1.

The first-degree relative group consisted of the parents, children, and siblings of type 1 diabetes patients. The siblings and children are followed within the T1D prediction programme Predia (www.predia.cz) and are regularly tested for anti-GAD65 and anti-IA2 autoantibodies. Out of this group we selected and analyzed 21 children with positive autoantibodies and 35 without any autoantibodies.

Thirty-nine patients in this subgroup were available for an analysis of genetic risk based on HLA class II haplotype associations. Relatives were HLA-typed using allele-specific primers [26] and we divided this subgroup based on the presence of high-risk and protective HLA haplotypes and alleles. In our study, siblings having one or two protective DQB1 allele were placed in the "low-risk" group (*DQB1*0602*, *DQB1*0503*, *DQB1*0301* and *DQA1*01–DQB1*0603*), whereas the remaining siblings were included in the "high-risk" group bearing genotypes *DQA1*05–DQB1*0201/DQA1*03–DQB1*0302* or *DQA1*05–DQB1*0201/X DQA1*03–DQB1*0302/X*, where X is none of protective alleles mentioned above [4].

Informed consent was obtained from all patients, patient guardians, and healthy donors prior to enrollment in this study.

Peripheral venous blood was collected from all donors into EDTA tubes. The staining protocol, gating strategy, and quantification of pDCs and mDCs have been described